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Mutations of *putP* that alter the lithium sensitivity of *Salmonella typhimurium*

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Summary

The *putP* gene encodes the major proline permease in *Salmonella typhimurium* that couples transport of proline to the sodium electrochemical gradient. To identify residues involved in the cation binding site, we have isolated *putP* mutants that confer resistance to lithium during growth on proline. Wild-type *S. typhimurium* can grow well on proline as the sole carbon source in media supplemented with NaCl, but grows poorly when LiCl is substituted for NaCl. In contrast to the growth phenotype, proline permease is capable of transporting proline via Na^+/proline or Li^+/proline symport. Therefore, we selected mutants that grow well on media containing proline as the sole carbon source in the presence of lithium ions. All of the mutants assayed exhibit decreased rates of Li^+/proline and Na^+/proline cotransport relative to wild type. The location of each mutation was determined by deletion mapping: the mutations cluster in two small deletion intervals at the 5′ and 3′ termini of the *putP* gene. The map positions of these lithium resistance mutations are different from the locations of the previously isolated substrate specificity mutations. These results suggest that Li^r mutations may define domains of the protein that fold to form the cation binding site of proline permease.

Introduction

Transport proteins must display a high degree of specificity in order to discriminate between different molecules presented to the cell (Saier, 1985). However, the precise mechanisms of substrate binding and translocation are not yet known for any transport protein. About 40% of the substrates transported by *Escherichia coli* and *Salmonella typhimurium* enter the cell by ion-driven transport systems (Wilson, 1978). The energy requirement for substrate accumulation in these systems is obtained either directly (i.e. proton symport) or indirectly (e.g. sodium symport) from the chemiosmotic gradient (Wilson, 1978). Specificity of ion-substrate cotransport requires unique binding sites for the substrate and for the specific counter-ion of the permease.

The main uptake route of proline in *S. typhimurium* and *E. coli* is via the proline permease encoded by the *putP* gene (Ratzkin and Roth, 1978). Proline transport is bidirectional, with a 1:1 stoichiometry of proline to Na^+ uptake (Chen and Wilson, 1986). Proline transport is obligately coupled to Na^+ or Li^+ cotransport; H^+ ions are not translocated by proline permease (Cairney *et al*, 1984). The great deal of biochemistry (Tristam and Neale, 1968; Rowland and Tristam, 1975; Wood and Zadworny, 1979; Stewart and Booth, 1983; Cairney *et al.*, 1984; Hanada *et al.*, 1985; Chen *et al.*, 1985; Chen and Wilson, 1986) and genetics (Ratzkin *et al.*, 1978; Wood and Zadworny, 1980; Dila and Maloy, 1986; Mogi *et al.*, 1986; Nakao *et al.*, 1987) done on proline permease in *E. coli* and *S. typhimurium* makes proline permease a good model system for studying the mechanism of ion-solute cotransport.

It is difficult to localize the active site of a permease by biochemical means. It is possible, however, to isolate rare missense mutations that alter the affinity of a permease for substrates: this allows the use of a direct genetic approach to identify the amino acids at or around the active site of a permease (Mieschendahl *et al.*, 1981; Brooker and Wilson, 1985; Dila and Maloy, 1986). For example, proline permease transports three substrates: proline, 3,4-dehydroproline, and azetidine-2-carboxylic acid. The most common type of *putP* mutant isolated displays a null phenotype. Null mutants are unable to transport any of these substrates. Null mutations can be isolated throughout the *putP* gene (Maloy, 1987). Substrate-specificity mutants of *putP* have been isolated which transport some but not all of the substrates (Dila and Maloy, 1986). Substrate specificity mutations cluster in three small regions of the *putP* gene. Substrate specificity mutations are thought to indicate amino acid residues involved in substrate binding and translocation (Myers *et al.*, 1987).

It should also be possible to isolate mutants with altered cation specificity. For example, some mutations in the *melB* gene in *E. coli* have been shown to affect specificity of H^+- and Li^+-coupled cotransport of melibiose (Shiota *et al.*, 1985). In this paper we report the isolation and characterization of *putP* permease mutants that display altered specificity for Li^+ as a coupling cation. Detailed analysis of these mutations should allow us to identify the

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amino acids at or near the cation binding site of proline permease.

Results

Lithium accumulation by proline permease is toxic

Although proline permease has been shown to couple proline transport directly to either sodium or lithium cotransport (Table 1; Cairney *et al.*, 1984; Tsuchiya *et al.*, 1985; Chen *et al.*, 1985), wild-type *S. typhimurium* LT2 grows very poorly on low-sodium medium with proline as the sole carbon source and 20 mM LiCl (phenotypically Li^s; Table 1). Cells grown on succinate, which is transported by H^+ symport (Kay, 1978), are not Li^s. However, when proline is added to cells growing on succinate + LiCl medium, growth stops, suggesting that lithium sensitivity is due to Li^+/proline symport (Table 1). If the *putP* gene is inactivated by a Tn*10* insertion, growth on succinate + LiCl medium is not impaired by the addition of proline. These data strongly suggest that the lithium sensitivity requires proline/Li^+ cotransport via proline permease.

Isolation of cation specificity mutants

We thought it might be possible to isolate rare *putP* mutations that alter the cation specificity of proline permease to allow growth on proline in the presence of LiCl (Li^r). In order to determine if it was possible to isolate Li^r mutants, we initially did general mutagenesis with nitrosoguanidine (NG) to identify all loci that could display this phenotype. A lawn of 10^8 cells of LT2 grown on MT + succinate medium was spread on MT medium + proline + LiCl (MTLP) and a few crystals of NG were placed in the centre of each plate. Large, fast-growing colonies that appeared were picked and patched on MTLP plates along with an unmutagenized LT2 control. Eighteen mutants that grew better than LT2 on MTLP plates were analysed further.

Linkage of lithium resistance mutations to putP

Putative 'cation specificity' mutants obtained by NG mutagenesis were tested for linkage of Li^r to the *put* operon. A phage lysate of TT1797 [*putP*$^+$ *zcc-7*::Tn*10*] was used to transduce the mutants to Tc^r. Transductants were screened for growth on MTLP TTC relative to the parent NG mutants and a TT1797 control. In each transduction, approximately 75% of the colonies were Tc^r Li^s. In order to verify linkage of the Li^r mutations to the *put* operon, phage lysates grown on the Li^r Tc^r progeny from the first cross were used to transduce MS71 (deleted for *putP*) to PSN^+ and scored for cotransduction of Li^r and Tc^r. Li^r was found to be 100% cotransducible with PSN^+ and very close linkage of Tc^r and Li^r was observed. From these experiments we concluded that all of the cation specificity mutations were tightly linked to the *putP* gene.

Table 1. Lithium accumulation by proline permease is toxic[a].

	putP$^+$		*putP*::Tn*10*	
Substrate/cation[b]	Pro transport	Growth	Pro transport	Growth
Pro-Na^+	+	+	–	–
Pro/Li^+	+	–	ND	–
Succ/Pro/Li^+	ND	–	ND	+
Succ/Li^+	ND	+	ND	+

a. The results shown are for LT2 (*putP*$^+$) and MS933 (*putP1102*::Tn*10*). ND, not determined.
b. MT medium was supplemented with the substrates and cations indicated, as described in *Experimental procedures*.

Localized mutagenesis of the put *operon*

Although nitrosoguanidine mutagenesis is a powerful tool in the search for rare classes of mutants, it can generate multiple mutations that may be hard to sort out during subsequent mapping experiments. Since all of the mutants obtained by nitrosoguanidine mutagenesis were linked to the *put* operon, we performed localized mutagenesis of TT1797. One of the virtues of localized mutagenesis is that it greatly facilitates the search for mutants in a particular region by restricting the number of transductants to be screened to those that are linked to a selectable marker (in this case, Tc^r from *zcc-7*::Tn*10* is approximately 80% linked to the *put* operon). Localized mutagenesis was performed with three mutagens in order to obtain a spectrum of point mutations. The mutagenized phage stocks were used to transduce LT2 to Tc^r, and the transductants were scored for Li^r. Li^r mutants were obtained approximately 10-fold less often than *putP* mutants displaying a null phenotype. The Li^r mutants presumably result from rare missense mutations that alter the cation specificity of proline permease.

In order to verify that the Li^r mutations were linked to Tc^r, phage lysates of the Tc^r Li^r mutants were used to transduce LT2 to Tc^r and the transductants were scored for Li^r. In every case, the linkage of Li^r to Tc^r was identical to that of bona fide *putP* mutants. If unlinked mutations were required for the Li^r phenotype, very few transductants would have simultaneously become Tc^r and Li^r.

Cations affect growth on proline as sole carbon source

To confirm the phenotypes observed on petri plates, growth rates of the mutants were measured under conditions that required cation-substrate cotransport. The

growth rate on proline as a sole carbon source was determined in three media: MT medium in the absence of supplemental cations, MT medium + 20 mM NaCl, and MT medium + 20 mM LiCl. The unmutagenized parental strain (LT2 or TT1797) grew well on MT + 0.1% proline (MTP) when supplemented with 20 mM NaCl (MTNP), grew slowly on unsupplemented MTP medium, and grew very poorly on MTP + 20 mM LiCl (MTLP) (Table 2, lines 1 and 2). A *putP* null mutant did not grow under any of these conditions (Table 2, line 3). The Li^r mutants grew up to 2.5-fold faster than the parental strain on MTLP (Table 2, lines 4–27). In contrast, growth of the Li^r mutants on MTNP was similar to that of the parental strain. Growth on MTP divided the mutants into two classes: one class grew as well as the parental strain on unsupplemented MTP medium (Table 2, lines 4–24), whereas the other class grew poorly on unsupplemented MTP medium (Table 2, lines 25–27).

Lithium resistance is specific for proline cotransport

We tested for possible pleiotropic phenotypes of the Li^r mutants by examining growth in MTL medium supplemented with carbon sources with known uptake pathways. Growth of the Li^r mutants on MTL + melibiose (Na^+ symport), succinate (H^+ symport), or glycerol (facilitated diffusion) was not stimulated relative to the unmutagenized parent (data not shown).

Deletion mapping

The properties of the Li^r mutants suggested that they map within the *putP* gene. In order to map the mutations within the *putP* gene, we carried out transductional crosses with donor phage grown on each of the mutants. The recipients for these crosses were deletion mutants unable to grow on proline as a sole nitrogen source (PSN^-) which divide the *putP* gene into 11 discrete intervals. The deletion mutants were transduced with phage lysates of the PSN^+ cation-specificity mutants selecting for growth on PSN plates as previously described (Dila and Maloy, 1986). PSN^+ transductants were picked and patched onto PSN plates along with donor Li^r colonies and the unmutagenized parent and incubated at 37°C. The PSN^+ patches were screened for coinheritance of Li^r by first replica printing onto noble agar plates followed by printing the noble agar plates onto MTLP TTC with a fresh velvet. The extra printing step was necessary to limit carry-over of Na^+, which otherwise obscured the results. When a mutation was crossed with a deletion that removes the corresponding sequence from the chromosome, 100% of the PSN^+ transductants inherited Li^r. However, when a mutation was crossed with a

Table 2. Effect of Na^+ and Li^+ on proline-dependent growth of the cation specificity mutants.

Strain	*putP* allele	Doubling time (h)[a]		
		Pro + Na^+	Pro + Li^+	Pro
1. LT2	wild type	2.0	11.3	4.9
2. TT1797	wild type	2.1	10.2	4.5
3. MS933	*putP1102*::Tn*10*	17.5	18.0	18.0
4. MS625	*putP1172*	2.4	6.3	5.1
5. MS627	*putP1174*	2.2	5.7	5.5
6. MS628	*putP1175*	2.6	5.1	5.8
7. MS629	*putP1176*	2.6	5.9	5.2
8. MS630	*putP1177*	2.0	6.1	5.6
9. MS631	*putP1178*	2.3	5.2	6.0
10. MS632	*putP1179*	2.2	5.4	6.1
11. MS633	*putP1180*	2.1	5.4	5.1
12. MS634	*putP1181*	2.0	6.2	6.3
13. MS635	*putP1182*	2.2	5.9	5.4
14. MS636	*putP1183*	2.0	4.1	5.3
15. MS637	*putP1184*	2.2	4.0	5.2
16. MS638	*putP1185*	2.4	3.4	4.8
17. MS639	*putP1186*	2.2	4.2	5.4
18. MS640	*putP1187*	2.1	4.4	5.6
19. MS641	*putP1188*	2.2	6.5	5.1
20. MS642	*putP1189*	2.0	4.0	4.9
21. MS643	*putP1190*	2.1	5.2	6.0
22. MS644	*putP1191*	2.4	3.6	5.1
23. MS646	*putP1193*	2.0	3.1	4.4
24. MS647	*putP1194*	2.2	3.2	4.5
25. MS626	*putP1173*	2.3	5.1	14.0
26. MS645	*putP1192*	2.0	3.5	10.2
27. MS648	*putP1195*	2.2	3.6	10.5

a. MT medium + 0.1% proline was supplemented with 20 mM NaCl (Na^+), 20 mM LiCl (Li^+) or was not supplemented with cations.

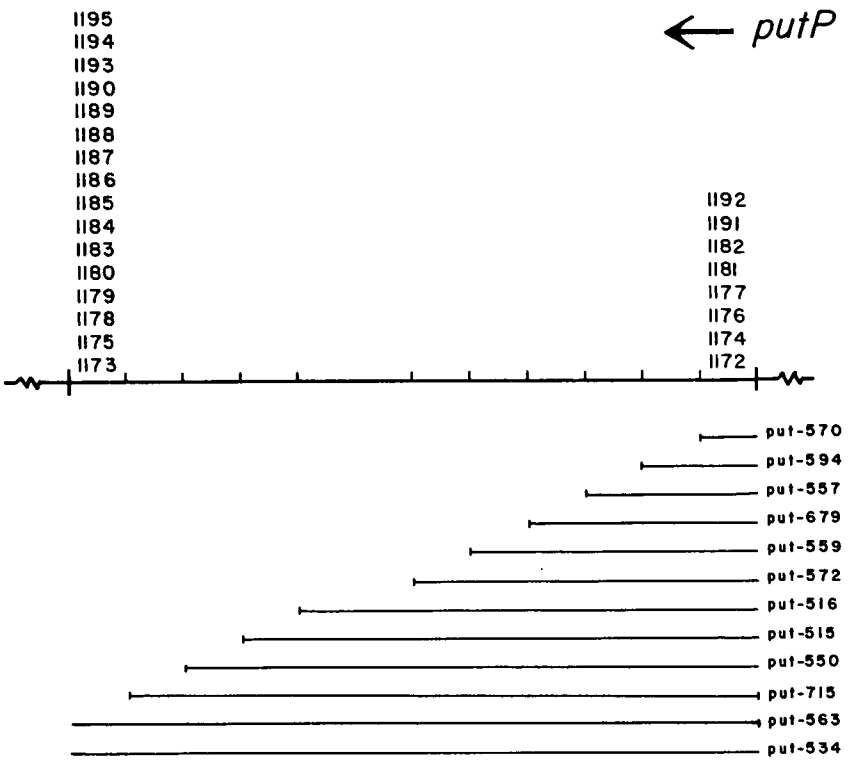

Fig. 1. Deletion map of the cation specificity mutations in *putP*. The map positions and allele numbers of the cation specificity mutations are indicated at the top, and the deletion mutations are shown below. The *putP* promoter is on the right-hand side of the diagram.

deletion that removes chromosomal sequences adjacent to, but not including, the corresponding chromosomal sequences, only 90–98% of the transductants inherited Li^r. In crosses with other deletions, less than 90% of the transductants inherited Li^r. The mutations clustered in two discrete deletion intervals at the 5′ and 3′ termini of the *putP* gene (Fig. 1).

Na^+/proline and Li^+/proline cotransport

In order to determine the activity of proline permease in the mutants, proline transport was measured with a fixed concentration of proline and various amounts of NaCl and LiCl. The initial velocity of proline transport by the mutants was lower than the wild type at every cation concentration tested (Table 3). In the transport media used, the concentration of contaminating NaCl (approximately 3 μM) was greater than the apparent K_m for Na^+, and thus we were unable accurately to determine the kinetics of Na^+/proline and Li^+/proline cotransport by the mutants (Cornish-Bowden, 1979). However, the decreased rates of lithium uptake by the mutants is consistent with the Li^r growth phenotype. In addition, three of the mutants assayed exhibit greater defects in Li^+/proline cotransport than in Na^+/proline cotransport relative to the wild type. These results indicate that the cation specificity of these mutants is altered.

Table 3. Li^r mutants are defective in Li^+/proline cotransport by proline permease.

		Proline Transport (nmol/min/mg protein)			
	putP	2.8 μM NaCl	9.2 μM LiCl		Defect
Strain	allele	Total	Total	Corrected[a]	Li^+/Na^+
TT1797	*putP*$^+$	62.0 (1.0)[b]	76.2	14.2 (1.0)[b]	1.0
MS644	*putP1191*	29.0 (2.1)	39.0	10.0 (1.4)	0.7
MS645	*putP1192*	17.5 (3.5)	19.4	1.9 (7.5)	2.1
MS647	*putP1194*	36.7 (1.7)	40.4	3.7 (3.8)	2.3
MS648	*putP1195*	42.0 (1.5)	43.5	1.6 (8.9)	5.9

a. Li^+/proline cotransport corrected for 2.8 μM contaminating NaCl by subtraction.
b. The numbers in parentheses show the defect in ion-driven proline transport relative to TT1797.

Discussion

Several lines of evidence indicate that proline transport is very similar in *E. coli* and *S. typhimurium*. Proline transport is coupled to the sodium gradient in both bacteria (Cairney *et al.*, 1984; Chen *et al.*, 1985) and the kinetics of Na^+/proline cotransport is identical. In addition, mutations in the *putP* structural gene in *E. coli* can be complemented by a plasmid carrying the *putP* gene from *S. typhimurium* (Hahn *et al.*, 1988). Li^+/proline cotransport has been previously demonstrated in *E. coli* (Chen *et al.*, 1985) and in this paper we show similar results for *S. typhimurium*. Furthermore, on low-Na^+ medium, Li^+ greatly inhibits growth of both *E. coli* and *S. typhimurium* on proline. We have used this phenotype as the basis of a selection for *putP* mutants with altered cation/proline cotransport.

Mutants that could grow on proline in a low Na^+/high Li^+ medium were initially obtained by nitrosoguanidine mutagenesis. Since mutations linked to the *putP* gene were sufficient to confer Li^r, we restricted our search by using localized mutagenesis near the *put* operon. We used three different mutagens (hydroxylamine, 2-aminopurine, and diethylsulphate) in order to generate a full complement of transition and transversion mutations. Using this approach, we obtained rare mutants that showed enhanced growth on proline in the presence of Li^+. The mutations conferring Li^r were found to be linked to the *put* operon and cotransduced with the *putP* gene. All of the mutants grew as well as the wild type on Na^+ + proline medium while acquiring the ability to grow in the presence of concentrations of Li^+ that inhibit the wild type (Table 2).

When grown on MT + proline medium without supplemental monovalent cations, the mutants fell into two phenotypic classes. MT medium has very low contaminating levels of Na^+ and Li^+. Trace levels of contaminating Na^+ were determined, by atomic-emission spectroscopy, to be 40 μM, with less than 2 μM contaminating Li^+. The K_T (Na^+) for proline permease in *E. coli* is reported to be 37 μM (Chen *et al.*, 1985) and the K_T (Na^+) in *S. typhimurium* appears to be less than 3 μM (R. Myers, unpublished observations). The largest class of Li^r mutants obtained grew as well as the wild type on MT + proline medium without added Na^+ or Li^+. This class of mutants may be defective in Li^+/proline symport while maintaining the ability to efficiently couple proline transport to the Na^+ electrochemical gradient. The second class of mutants grew much slower than the wild type on MT medium without Na^+ or Li^+. These mutants may be defective in both Na^+/proline and Li^+/proline symport, possibly because of a general monovalent cation-binding defect.

In order to assess transport activity in the mutants, initial rates of proline transport were measured at several concentrations of NaCl and LiCl. In every mutant assayed, Li^+/proline cotransport was decreased relative to the wild type. Taken together with the growth phenotype, these results suggest that these Li^r mutants alter the cation specificity of proline permease.

The simplest interpretation of our results is that Li^r mutations alter residues that contribute to the cation-binding domain of proline permease. If this is correct, the mutations would be expected to cluster in regions of the *putP* structural gene that correspond to cation-binding domains of proline permease. To test this hypothesis, we mapped the Li^r mutations with a nested set of deletions of the *putP* gene. The mutations were found in only two of the eleven deletion intervals of the *putP* gene (Fig. 1). Both classes of mutations mapped in the same deletion intervals. However, the Li^r mutations map in different intervals than previously isolated substrate specificity mutations (Dila, 1986). These results suggest that the active site of proline permease may be divided into two discrete functional domains: a substrate-binding domain and a cation-binding domain.

This *in vivo* genetic approach allowed us to isolate a large number of mutations that alter residues at or near the active site of proline permease. We plan to determine the DNA sequence of the substrate specificity mutations and cation specificity mutations to identify the amino acids that comprise the active site. By combined genetic and molecular approaches we hope to dissect the active site of proline permease to gain insight into the general mechanism of ion/solute cotransport.

Experimental procedures

Bacterial strains

All strains were derived from *S. typhimurium* LT2. The genotypes of the strains used are shown in Table 4.

Media and growth conditions

Nutrient broth with 0.5% NaCl was used as a rich medium. Three different minimal media were used: NCE (Ratzkin and Roth, 1978), a medium lacking both carbon and nitrogen sources (NCN) (Berkowitz *et al.*, 1968), and a low-sodium medium lacking carbon (MT) (Shiota *et al.*, 1985). MT medium was supplemented with 20 mM LiCl or 20 mM NaCl. Carbon sources were added to minimal media at the following concentrations: 0.1% proline, 0.6% potassium succinate, 0.4% glycerol, and 0.34% melibiose. NCN medium with succinate and 0.2% L-proline (PSN) was used to check the ability to grow on proline as sole nitrogen source. Growth of the Li^r mutants was followed in a Klett-Summerson colorimeter with a green filter. Tetracycline HCl (Tc) was added to rich medium at 25 μg ml^{-1} and to minimal media at 10 μg ml^{-1}. When TTC (2,3,5-triphenyl-2H-tetrazolium chloride) was used as

Table 4. Bacterial strains.

Strain	Geonotype	Source[a]
LT2	wild type	J. Roth
TT1797	*zcc-7*::Tn*10*	Menzel and Roth (1981)
MS933	*putP1102*::Tn*10*	Lab collection
TR2619	Δ*(putPA)516 pyrC7*	Ratzkin and Roth (1978)
TR3400	Δ*(putPA)515 pyrC7*	Ratzkin and Roth (1978)
TR4921	Δ*(putPA)534*	Ratzkin and Roth (1978)
TR4937	Δ*(putPA)550*	Ratzkin and Roth (1978)
TR4942	Δ*(putPA)555*	Ratzkin and Roth (1978)
TR4944	Δ*(putPA)557*	Ratzkin and Roth (1978)
TR4946	Δ*(putPA)559*	Ratzkin and Roth (1978)
TR4950	Δ*(putPA)563*	Ratzkin and Roth (1978)
TR4957	Δ*(putPA)570*	Ratzkin and Roth (1978)
TR4959	Δ*(putPA)572*	Ratzkin and Roth (1978)
TR4981	Δ*(putPA)594*	Ratzkin and Roth (1978)
TR5023	Δ*(putPA)715*	Ratzkin and Roth (1978)
MS625	*putP1172*	This study
MS626	*putP1173*	This study
MS627	*putP1174*	This study
MS628	*putP1175*	This study
MS629	*putP1176*	This study
MS630	*putP1177*	This study
MS631	*putP1178*	This study
MS632	*putP1179*	This study
MS633	*putP1180*	This study
MS634	*putP1181*	This study
MS635	*putP1182*	This study
MS636	*putP1183*	This study
MS637	*putP1184*	This study
MS638	*putP1185 zcc-7*::Tn*10*	This study
MS639	*putP1186*	This study
MS640	*putP1187*	This study
MS641	*putP1188*	This study
MS642	*putP1189*	This study
MS643	*putP1190*	This study
MS644	*putP1191 zcc-7*::Tn*10*	This study
MS645	*putP1192 zcc-7*::Tn*10*	This study
MS646	*putP1193 zcc-7*::Tn*10*	This study
MS647	*putP1194 zcc-7*::Tn*10*	This study
MS648	*putP1195 zcc-7*::Tn*10*	This study

a. Strains constructed by Menzel and Roth (1980) and Ratzkin and Roth (1978) were obtained from J. Roth, Department of Biology, University of Utah, Salt Lake City, USA.

a growth-rate indicator (Bochner and Savageau 1977), it was added to MT medium at 2.5 mg ml^{-1}. Bacto agar was added to solid media at 1.5%. Noble agar was substituted for Bacto agar in PSN and MT media to minimize contaminating nitrogen and NaCl.

Genetic techniques

All genetic manipulations used P22 HT105/1 *int-201*, a high-frequency generalized transducing phage that cannot form stable lysogens (Schmeiger, 1972). Preparation of phage lysates and transductions were done as previously described (Hahn and Maloy, 1986). Mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was performed by placing a few crystals of NG in the centre of an MT + proline + 20 mM LiCl (MTLP) plate spread with 10^8 cells of LT2. Localized hydroxylamine (HA) mutagenesis was performed *in vitro* as described previously (Hong and Ames, 1971; Davis *et al.*, 1980). Localized mutagenesis with 2-aminopurine (AP) and diethylsulphate (DES) was performed by mutagenizing TT1797 (*zcc-7*::Tn*10*, Tn*10* 80% linked to *putP*) *in vivo* (Roth, 1970), and then phage lysates grown on mutagenized cells were used to transduce LT2 to Tc^r. Only one mutant was isolated from each AP- or DES-treated phage stock in order to avoid isolating siblings. Potential Li^r mutants were obtained by screening for 'early' red colonies on MTLP Tc TTC indicator plates under a dissecting microscope. Likely candidates were streaked out on MTLP plates and all colonies that grew faster than the unmutagenized parent were characterized more fully. Proline permease mutants with a null phenotype were isolated at $10^{-2} - 10^{-3}$ of total Tc^r colonies plated. In contrast, Li^r mutant were isolated about 10-fold less frequently for each mutagen tested.

Proline transport

Cells were grown in NCE + succinate + proline to late log phase, washed twice in MT medium, resuspended in 0.5 volume MT + 1 mM dextrose + 50 μg ml^{-1} chloramphenicol, and held on ice until assayed for active transport. A 0.2 ml reaction mix containing MT medium, 1 mM dextrose, 50 μg ml^{-1} chloramphenicol, 45 μM L-proline, 5 μM L-[U-^{14}C]-proline (285 mCi $mmol^{-1}$; Amersham, Arlington Hts., Illinois), and NaCl or LiCl at several different concentrations was added to 7 ml plastic mini-vials. Prior to assaying transport, cells (0.25 mg cells dry weight ml^{-1}) were swirled on a rotary shaker (100 RPM) for 15 min at room temperature to starve for proline. Then 0.2 ml of starved cells was rapidly added to the mini-vials containing the reaction mix to initiate the transport assay. At the appropriate time, 5 ml stopping buffer (5 mM 2[*N*-morpholino]ethanesulphonic acid, 300 mM KCl, 2 mM $HgCl_2$, pH 7.0) was rapidly added to the mini-vials. Uptake of proline was measured over a 15 sec period at 5 sec intervals. The 0 sec time point was obtained by diluting starved cells directly into reaction mix plus stopping buffer. Transport reactions were passed through 0.45 μm cellulose nitrate filters (Sartorius) prewet with stopping buffer less than 10 min after transport was halted, and washed once with 5 ml stopping buffer. All transport assays were carried out at least three times.

Chemicals

Certified grade LiCl and succinic acid were purchased from Fischer Scientific (Fair Lawn, New Jersey). Tris (hydroxymethyl)-aminomethane (ultrapure) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Indiana). Media and agar were obtained from Difco Laboratories (Detroit, Michigan). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, Missouri).

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